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THYROID HORMONE INDICES IN CHILDREN INFECTED WITH GIARDIASIS AND AMOEBIASIS IN KARBALA, IRAQ

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ABSTRACT

Background: *Giardia lamblia* and *Entamoeba histolytica* are important intestinal protozoa that cause public health problems in most developing countries. Both causing diarrhea with the majority of patients being children. Up to now few reports about hormonal alterations induced by *G.lamblia* and *E.histolytica* infection have been described. We study the alterations in the levels of Free Thyroxine and Free Triiodothyronine in blood of children infected with Giardiasis and Amoebiasis

Objective: To evaluate thyroid hormone indices including the free thyroxin (FT4) and free triiodothyronine (FT3) in children suffering from diarrheadue to *G.lamblia* and *E.histolytica*, and compared the results with data obtained from a sex and age matched parasite-free control group.

Design: This work was a hospital based cross-sectional descriptive study conducted in Karbala teaching hospital for children (Karbala, Iraq) during June to December, 2012.Stool specimens were collected from symptomatic 184 child, aged 6 months to 14 years attending the out patients clinics and complaining from diarrhea confirmed to be positive for *G.lamblia* and *E.histolytica* in different stages of the parasites. The free thyroxin (FT4) and free triiodo thyronine (FT3) measurement done for the diarrheal group by menividus technique and compared the results with data obtained from 27, sex and age matched parasite-free children as a control group.

Results: In this study was found significance difference in the dependent means at ($P \leq 0.05$) of the level of number Lymphocyte between children infected with protozoa(*E. histolytica* and *G. lamblia*) in compare with the normal groups and significant changes in the level of hormones Triiodothyronine (T3) and Thyroxin(T4) in blood sample from children infected with *E. histolytica* and no significant changes in the level of thyroid stimulating hormones (TSH) between children infected with protozoa(*G. lamblia* and *E. histolytica*) compare with the normal group. but there is significant changes in level of hormone in chronic stage of *G. lamblia* when compered between stage of parasite in the infected children.

KEYWORDS: *Giardia lamblia* and *Entamoeba histolytica*, Thyroxine, Triiodothyronine

INTRODUCTION

The prevalence of amoebiasis and giardiasis varies in population of countries and between areas with different socioeconomic conditions. Some up to 50% of the population is affected in regions with poor sanitary conditions [1, 2].

In Iraq literatures showed different incidence levels of *G. lamblia* and *E. histolytica*/ *E.dispare* infection in different age groups [3, 4, 5] as well as some papers focused on sex and age related prevalence especially in children [6,7]. We need more descriptive data to update our knowledge about these two parasites especially in children due to a

considerable medical and public health problems in developing countries, like diarrhea, blood loss, malabsorption and growth retardation that can be associated with these intestinal infections. [8,9].

Giardiasis may cause acute or chronic diarrhea, dehydration, abdominal discomfort, and weight loss. Enteric infection with *G. lamblia* is responsible for decreased absorption of electrolytes, glucose and fluid, at least in part because of diffuse epithelial microvillus shortening, which may be combined or not with villous atrophy [10]. There was a relationship between protein kinase C in intestine, secretion of Na^+ and $Cl^-$and *G.lamblia* effect [11]. However, the role of Giardia virulence factors, such as proteinases and lectins, in modulating secretory responses needs to be fully determined. Together these abnormalities lead to the malabsorption and maldigestion that ultimately cause failure to thrive in children.

Also 10% of people infected with *E. histolytica* actually develop symptoms. In those who present with invasive disease, there is an initial ulceration of the intestinal epithelium due to the expression of several cysteine proteases, which degrade the extracellular matrix, and due to amoebapores which is an important virulence factor of *E. histolytica* that cause cell lysis[12,13].This results in cell detachment, reducing the absorptive surface of the intestine. In addition to changes in tight junction activity, there are also changes in ion transport. Analysis of amebic lysates suggests that there are two distinct actions on Cl^- secretion: there is a Ca^{2+} dependent process, which is active in ileum and a cAMP-dependent process, which is active in colon. The Ca^{2+}-dependent response is only active on the serosal surface, suggesting that these mechanisms are not activated until the epithelial layer is breached [14,15].

The thyroidhormones, triiodothyronine (T_3)and Thyroxin (T_4),are tyrosine based hormones produced by the thyroid gland that are primarily responsible for regulation of metabolism. The thyroid hormones act on nearly every cell in the body. They act to increase the basal metabolic rate, affect protein synthesis, help to regulate long bones growth (synergy with growth hormone) and neural maturation, and increase the body's sensitivity to catecholamines (such as adrenaline)by permissiveness[16,17].

The thyroid hormones are essential to proper development and differentiation of all cells Of the human body. These hormones also regulate the protein, fat, and carbohydrate metabolism, stimulate vitamin metabolism and also affect the Na (+)-K+ pump. The long-term regulation exerted by thyroid hormone and aldoster one is mediated by changes in gene expression [18]. The sodium-iodide symporter (Na^+/I^- symporter) plays pivotal role in iodide condensation that is an indispensable step in the biosynthesis of the thyroid hormone pronounced effect of thyroid dysfunction in young age group is on growth and development but it also leads to metabolic abnormalities similar to those in adults. Thyroid hormones increase oxygen consumption, stimulate protein synthesis, affect carbohydrate, lipid and vitamin metabolism, and influence activity of other enzyme systems and growth factors. In the brain they promote cell growth, cell differentiation and induce hypothyroidism remains unrecognized during early childhood, physical growth potential can also be affected. Hyperthyroidism can induce rapid linear and skeletal growth and maturation. Approximately 100mg of thyroxin (T4) is secreted by the thyroid gland daily and about 90mg of iodide (15mg/kg) is the recommended daily intake during infancy and childhood with higher requirements in preterm. [13,19,20].

MATERIALS AND METHODS

This work was a hospital based cross-sectional descriptive study conducted in Karbala teaching hospital for children (in Karbala, Iraq) during June through December, 2012.

In this study we examined 5670 children attending the Hospital. Stool specimens were collected from symptomatic patients attending the out patients clinics and complaining from diarrhea. Those patients with diarrhea and confirmed to be stool exam positive for *G. lamblia* and/or *E. histolytica*were 184, and compared with 27 child without diarrhea and were negative for the two protozoa. The parasite detection was performed by conventional microscopy on two types of direct wet film preparations for each sample at the same time, one slide by using normal saline for detecting the motility of Trophozoites and the other by Lugol's iodine 5% and buffer methylene blue in slide for demonstrating the internal protozoal structures and recognize red blood cells inside parasite.

All smears were examined by experienced technician for the presence of *G. lamblia* and *E. histolytica* cysts and trophozoites.

Blood samples (5ml) were collected by using disposable syringes, 3ml was put in a plane tube (without EDTA) for serological analysis. For measuring the level of hormones used minivides analytic instrument and kit for minivides to measure the hormones (T3,T4,TSH) from (Bio merieux company) and another 2ml of blood put in tube with EDTE by Automated Hematology Analyzer to measure lymphocytes.[14,15].

Statistically T-test and Chi-squared were applied to find the significant difference between the data. P value (<0.05) were considered significant, also using Students-t-test for difference between two independent means at 0.05 level.

All participants were informed about the study protocol and consent was obtained from each of them.

RESULTS

In this study we examined184 patients with diarrhea and confirmed to be stool positive for *G. lamblia* and *E. histolytica* and 27 children without diarrhea and were negative for the two protozoa with different age groups range from below one year to less than 10 years.

The table 1 show that there are significant different changing in number of Lymphocyte in blood of children infected with the protozoa *G. lamblia* (0.034 P value) and *E. histolytica* (0.004P value) compared with the normal group. There was increased number of actives lymphocyte in children infected with *G. lamblia* and *E. histolytica* parasite.

Table 1: The Effect of the Type of Parasite (*G. lamblia*and *E. histolytica*) on Lymphocyte Count (L) Compared with Healthy Children

		E.histolytia	***G.lamblia***	**Normal**
L	No	101	83	27
	Mean±SD	29.38±14.19	33.34±15.50	48.98±18.94
	Standard Error of Mean	1.539	1.766	8.472
	Mode	30.00	10.00	34.00
	Range	1.05-69.0	7.0-68.0	34.0-79.0
	Percentile 05^{th}	6.67	10.0	34.0
	25^{th}	19.0	22.0	35.0
	50^{th} (Median)	30.0	32.0	40.9
	75^{th}	37.0	44.4	56.0
	95^{th}	55.0	64.0	79.0
	99^{th}	69.0	68.0	79.0
P value compared to Healthy control		0.004*	0.034*	-
P value compared to *G lamblia*		0.091	-	-
P value comparing All		0.009#		
*Significant using Students-t-test for difference between two independent means at 0.05 level.				
#Significant using ANOVA test for difference among independent means at 0.05 level.				

The results in table 2 showed that there are significantly different (decrease) in level of Triiodothyronine (T3) hormones at P value 0.0001 in blood of Children infected with protozoa *E. histolytica* compared with the normal group and no significant different in level of hormone in sample of children infected with *G. lamblia* parasites compared with normal group and there is significant different (0.0001 P value) when compares between the level of Triiodothyronine (T3) hormones in blood samples of children infected with *E. histolytica* and those infected with *G. lamblia*

Table 2: The Level of Triiodothyronine (T3) Hormones in Patient with (*G.lamblia* and *E.histolytica*) Infection Compared with Healthy Children

		E.histolytic	*G.lamblia*	Normal
T3	No	101	83	27
	Mean±SD	1.30±0.57	1.74±0.64	1.99±0.63
	Standard Error of Mean	0.057	0.070	0.121
	Mode	1.2	1.5	1.6
	Range	0.1-2.7	0.6-4.0	1.0-3.3
	Percentile 05^{th}	0.4	1.1	1.0
	25^{th}	1.0	1.3	1.6
	50^{th} (Median)	1.2	1.6	1.9
	75^{th}	1.7	2.0	2.5
	95^{th}	2.2	2.9	3.0
	99^{th}	2.6	4.0	3.3
P value compared to Healthy control		0.0001*	0.085	-
P value compared to G lamblia		0.0001*	-	-
P value comparing All		0.0001#		
*Significant using Students-t-test for difference between two independent means at 0.05 level.				
#Significant using ANOVA test for difference among independent means at 0.05 level.				

The results in table 3 showed that there are significantly different (decrease) in level of Thyroxin (T_4) hormones at P value (0.006) in blood of children infected with protozoa *E. histolytica* compare with the normal group and no Significant different in level of hormone in sample of children infected with *G. lamblia* parasites compared with normal group and there is significant different (0.010 P value) when compares between the level of Thyroxin (T_4) hormones in blood samples of children infected with *E. histolytica* and those infected with *G. lamblia*.

Table 3: The Level of Thyroxin (T4) Hormones in Patient with (*G.lamblia* and *E.histolytica*) Infection Compared with Healthy Children

		E.histolytica	*G lamblia*	Normal
T4	No	101	83	27
	Mean±SD	91.21±20.99	98.44±15.62	103.24±15.91
	Standard Error of Mean	2.089	1.714	3.061
	Mode	80	100	98
	Range	1-139	66-142	84-159
	Percentile 05^{th}	72	75	87
	25^{th}	80	88	97
	50^{th} (Median)	89	98	100
	75^{th}	100	105	106
	95^{th}	130	130	146
	99^{th}	137	142	159
P value compared to Healthy control		0.006*	0.170	-
P value compared to G lamblia		0.010*	-	-
P value comparing All		0.002#		
*Significant using Students-t-test for difference between two independent means at 0.05 level.				
#Significant using ANOVA test for difference among independent means at 0.05 level.				

The results in table 4 showed that the number of children, mean, P- value control, effect of the changing of type of parasite (*G.lambliaand E.histolytica*) on thyroid stimulating hormones (TSH) compared with healthy children. There was no significant changes between two independent means at 0.05 level changes in the level of thyroid stimulating hormones (TSH) hormones in blood of children infected with protozoa(*G. Lambliaand E.histolytica*) compare with the normal group and with each other.

Table 4: The Level of Thyroid Stimulating Hormones (TSH) in Patient with (*G.lamblia* and *E.histolytica*) Infection Compared with Healthy Children

		E.histolytica	***G.lamblia***	**Normal**
TSH	No	101	83	27
	Mean±SD	2.70±3.79	2.64±2.61	1.80±1.14
	Standard Error of Mean	0.377	0.287	0.219
	Mode	1.00	2.00	0.90
	Range	0.17-31.0	0.50-22.0	0.60-4.70
	Percentile 05^{th}	0.70	0.80	0.60
	25^{th}	1.00	1.40	0.90
	50^{th} (Median)	1.80	2.10	1.50
	75^{th}	3.00	2.80	2.00
	95^{th}	6.00	6.00	4.00
	99^{th}	21.00	22.00	4.70
P value compared to Healthy control		0.228	0.112	-
P value compared to G lamblia		0.893	-	-
P value comparing All		0.401		
*Significant using Students-t-test for difference between two independent means at 0.05 level.				
#Significant using ANOVA test for difference among independent means at 0.05 level.				

The results in table 5 showed that there are significantly difference (decrease) in (0.034 p value) in the level of thyroid stimulating hormones (TSH) hormones between children infected with protozoa *G. lamblia* (Cyst stage) compare with group of children infected with *E. histolytica* in all phases of the parasite. But as shown in table 4 there is no significant in level of TSH hormone in sample of children infected with *G. lamblia* parasites compared with normal group, also table 5 show the significant changes between the two independent means at 0.05 level changes (0.034 p value) in number of Lymphocyte in children infected with protozoa *E. histolytica* parasite (trophozoite stage) comparing with sample of children infected with *G. lamblia* in all phases of the parasite.

Table 5: The Level of (T3, T4, TSH, Lymphocyte) According to the Phase of Parasite (P-LCP) in Children Infected with *Giardia lamblia* and *Entamoebahistolytica*

Phase of parasite			**Parameter**	**Type of parasite**
P-LCR (Cyst)+(Troph)	**P-LCR (Troph)**	**P-LCR (Cyst)**		
0.384	0.08	0.178	T3	***E.histolytica***
0.08	0.44	0.175	T4	
0.54	0.73	0.16	TSH	
0.029	$_{\varphi\varphi}$0.043	0.902	L	
0.458	0.983	0.359	T3	***G.lamblia***
0.693	0.673	0.75	T4	
0.942	0.197	$_{\varphi\varphi}$0.034	TSH	
0.74	0.577	0.552	L	
$_{\varphi\varphi}$Significant using Students-t-test for difference between two independent means at 0.05 level.				

DISCUSSIONS

Analytical results including the number of children, mean, P- value and percentage changing in level of

Thyroxin(T4), Triiodothyronine (T3), thyroid stimulating hormones (TSH), in blood sample of children infected with parasite (*G. lamblia* and *E.histolytica*) compared with healthy children.

Relative lymphocytosis occurs when there is a higher proportion (greater than 40%) of lymphocytes among the total count of white blood cells, while the absolute lymphocyte count is normal when less than 4000 per microliter. The results of this study as shown in table 1 that there are significant changes in level of lymphocyte in children infected with *G. lamblia* and *E. histolytica* compared with the normal group, these changes might be due to an increase in the number of actives lymphocyte as a response to the infection, this result agree with a research **[**14] were found an increase the percentage of lymphocyte number when patient infected with *G. lamblia,* but another study[15] have different visions about no significant changes in number of lymphocyte in infected children with *G. lamblia* when compare with group of children infected with *En. histolytica,* all these visions have same discussion that the response of cellular immune system to the infection may need time and virulent factors to have active stimulation, so the child infected with *E. histolytical* need shorter time to increase the level of lymphocyte than *G. lamblia*. in Table 2, 3, 4 We see there are significant low levels of thyroid hormones T3, T4 in children infected with *E. histolytical* and no significant changes in level of hormone TSH, but table 5 showed significant changes of TSH in chronic states (cystic stage) in children infected with *G. lamblia* and no significant when compared with the stage of *E. histolytical* parasite.

In diarrhea there is body water and electrolyte loss including, sodium, calcium and potassium, many researches showed, that these disturbances will affect the function of osmotic regulations in the body and have a role on thyroid hormone stimulation across the plasma membrane of cells in all body[16, 17, 18, 19, 20, 21]. The cause of the depression in total serum T3 values with nonthyroidal illness is considered to be due to decreases peripheral tissue conversion of T4 to t3 whereas the low total T4 values may result from not fully identified circulating T4 binding inhibitor, which interferes with the binding of T4 to the serum carrier proteins …. Concomitant with clinical recovery, total serum T4 concentrations rapidly returned to the normal range, presumably indicating removal of the putative T4 binding inhibitor. [22]

Because under normal conditions, the intracellular concentrations of sodium and potassium are roughly the reverse of the corresponding concentrations in the extracellular fluid, there is a constant net inward leak of sodium ions and outward leak of potassium ions that in a steady state must be counter-balanced by active sodium and potassium transport in directions opposite to these passive fluxes if normal intracellular ion concentrations are to be maintained. On the basis of these findings about relationship between sodium and thyroid hormones it agreed with [16, 23, 24, 25**,** 26]

CONCLUSIONS

Infection with (*G. lamblia* and *E. histolytica*) produces alterations in thyroid function. There are significantly decrease in level of Triiodothyronine (T3) in children infected with *E. histolytica* as well as in sample of children infected with *G. lamblia*. There are significantly decrease in level of Thyroxin (T_4) hormones in blood of children infected with *E. histolytica* but not with *G. lamblia*. There was no significant changes in the level of thyroid stimulating hormones (TSH) hormones in blood of children infected with protozoa (*G. Lamblia* and *E.histolytica*) compared with the normal group.

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